

Kindly replace the paragraph on page 2, line 37 to page 3, line 3 with the following:

-- These repetitive motifs (SEQ ID NO: 1) of 17 amino acids are represented by the
formula:

Leu-Ala-Lys-Glu-Lys-Leu-Gln-X-Gln-Gln-Ser-Asp-Leu-Glu-Gln-Glu-Arg

in which X is Glu or Gly. --.

Kindly replace the paragraphs on page 3, line 15 to page 4, line 14 with the
following:

BRIEF DESCRIPTION OF THE DRAWINGS

Reference will be made in what follows to the Figures in which:

- Figure 1 (SEQ ID NO: 31) presents a recombinant protein of the invention of 316 amino acids, designated hereafter as antigen 536 or protein LSA-R-NR,
- Figure 2 (SEQ ID NO: 32) provides the nucleotide sequence of one of the recombinant nucleic acids studied (clone DG536) and which codes for the polypeptide LSA-R-NR,
- Figure 3 (SEQ ID NO: 24) presents a polypeptide of the invention of 151 amino acids, designated hereafter as antigen 729S,
- Figure 4 (SEQ ID NO: 33) corresponds to the nucleotide sequence of the clone DG729S which codes for the polypeptide of figure 3 (EcoRI linkers in bold type),
- Figure 5 presents the polypeptide sequences (SEQ ID NOS: 23 and 26-28) of the antigens LSA-Ter, 729S-NRI, 729S-NRII, 729S-Rep,

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- Figure 6 (SEQ ID NO: 34) presents the 5' end of the nucleotide sequence of the LSA gene,
 - Figures 7A-7C (SEQ ID NOS: 35-37) presents the coding sequence of the 5' end of the LSA gene and the corresponding polypeptide sequence,
 - Figure 8 (SEQ ID NO: 38) describes the 3' end of the LSA gene,
 - Figures 9A-9D (SEQ ID NOS: 39-42) gives the sequence of the 3' end of the LSA gene as well as the corresponding polypeptide sequence,
 - Figures 10A-10D (SEQ ID NOS: 43-46) repeats the sequences given in Figures 9A-9D, up to the termination codon stop and the terminal amino acid.

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Thus, the present invention relates to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in Figures 9A-9D or Figures 10A-10D, and corresponds to the 3' end of the LSA gene.

More particularly, the subject of the invention is any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is

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represented by all or part of the sequence of the last 279 amino acids shown in Figures 10A-10D, this amino acid sequence being optionally preceded by all or part of one or more of the sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula: --.

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Kindly replace the paragraph on page 5, line 6 to line 18 with the following:

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-- Consequently, the invention relates more particularly to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 19):

RKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPS
ENERGYYPHQSSLPQDNRGNSRDSKEISIEKTNR
ESITTNVEGRRDIHKHLEEKDGSIKPEQKEDKS

this amino acid sequence being optionally preceded by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula: --.

✓
Kindly replace the paragraphs on page 6, line 16 to line 34 with the following:

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-- The invention relates more particularly to any polypeptide characterized by all or part of the following amino acid sequence (SEQ ID NO: 20):

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BS
LQEQQRDLEQRKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPSENERGY
IPHQSSLPQDNRGNSRDSKEISIIIEKTNRESITTNVEGRRDIHKHGLEEKD
G
SIKPEQKEDKS

A preferred polypeptide of the invention is represented by all or part of the following amino acid sequence (SEQ ID NO: 21):

DTKKNLERKKEHGDILAEDLYGRLEIP

(this polypeptide being designated hereafter by the expression LSA-NR (LSA-non-repeated), or also by any sequence derived from the preceding sequence and modified by the substitution of maximally 40% of the amino acids while retaining its physiological activity such as the induction of a response of the T lymphocytes, in particular the cytotoxic T lymphocytes.

Another particularly preferred polypeptide of the invention is characterized by all or part of the following amino acid sequence (SEQ ID NO: 22):

ERRAKEKLQEQQRDLEQRKADTKK

(this polypeptide being designated hereafter by the expression LSA-J, or LSA-junction, since it overlaps the repetitive part and the non-repetitive part of the molecule shown in Figure 1). --.

Kindly replace the paragraphs on page 7, line 1 to line 27, with the following:

-- Another preferred peptide, designated LSA-TER, is the following (SEQ ID NO:

23):

NSRDSKEISIIIEKTNRESITTNVEGRRDIHK

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These last three polypeptides are more particularly useful on account of the amphipaticity which characterizes them, and because of their three-dimensional conformation according to the predictions made by the procedure of Chou and Fassmann.

The subject of the invention is also any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitope(s) characteristic of a protein produced at the sporozoite, hepatic and blood (erythrocytic) stages of P. falciparum, and more particularly bearing one or more T epitopes, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 24):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHNVEEKVE
ESVEENDEESVEENVEENVEENDDGSVASSVEESIASSVDESIDSSIEENVAP
TVEEIVAPTVEEIVAPSVVEKCAPSVVEESVAPSVVEESVAEMLKER

shown in Figure 3 and designated hereafter as the polypeptide 729S.

More particularly, the subject of the invention is the amino acid sequence derived from the preceding sequence and characterized by all or part of the following amino acid sequence (SEQ ID NO: 25):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQH

According to another advantageous embodiment of the invention, sequences of interest derived from the amino acid sequence of the polypeptide 729S are the following (SEQ ID NOS: 26-28):

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- DELFNELLNSVDVNGEVKENILEESQ,
- LEESQVNDDIFSNSLVKSVQEQQHNV,
- VEKCAPSVEESVAPSVEESVAEMLKER. --.

Kindly replace the paragraphs starting on page 8, line 1 to line 16 with the following:

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-- The subject of the invention is also any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in Figures 7A-7C

Consequently, the subject of the invention is more particularly any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the sequence of the first 153 amino acids shown in Figures 7A-7C, this amino acid sequence being optionally followed by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula: --.

Kindly replace the paragraph starting on page 9, line 6 to page 10, line 6 with the following:

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-- The invention also relates to any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence comprises successively:

- all or part of the sequence of the first 153 amino acids shown in Figures 7A-7C,
- optionally, all or part of one or more of the sequences of 17 amino acids (SEQ ID

NOS: 2-18) of formula:

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X₁DLEQX₂RX₃AKEKLQX₄QQ
QX₁DLEQX₂RX₃AKEKLQX₄Q
QQX₁DLEQX₂RX₃AKEKLQX₄
X₄QQX₁DLEQX₂RX₃AKEKLQ
QX₄QQX₁DLEQX₂RX₃AKEKL
LQX₄QQX₁DLEQX₂RX₃AKEK
KLQX₄QQX₁DLEQX₂RX₃AKE
EKLQX₄QQX₁DLEQX₂RX₃AK
KEKLQX₄QQX₁DLEQX₂RX₃A
AKEKLQX₄QQX₁DLEQX₂RX₃
X₃AKEKLQX₄QQX₁DLEQX₂R

Out
BS

$RX_3AKEKLQX_4QQX_1DLEQX_2$

$X_2RX_3AKEKLQX_4QQX_1DLEQ$

$QX_2RX_3AKEKLQX_4QQX_1DLE$

$EQX_2RX_3AKEKLQX_4QQX_1DL$

$LEQX_2RX_3AKEKLQX_4QQX_1D$

$DLEQX_2RX_3AKEKLQX_4QQX_1$

in which:

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Cal
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° X₁ is "Ser" or "Arg",

° X₂ is "Glu" or "Asp"

° X₃ is "Arg" or "Leu"

° X₄ is "Glu" or "Gly"

- and all or part of the last 279 amino acids shown in Figures 10A-10D. --.

Kindly replace the paragraph on page 12, line 1 to line 22 with the following:

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-- The invention also relates to any sequence of nucleotides which codes for a polypeptide identical with, or one similar from the point of view of both structure and antigenic properties to, those of the invention, this sequence being capable of hybridizing with all or part of the nucleotide sequence defined by the nucleotides situated at the positions 597 to 949 of figure 2, or with all or part of the nucleotide sequence of Figure 4 or the sequences complementary to these latter, under the following conditions:

- pre-treatment (pre-hybridization) of the nitrocellulose filter supporting the nucleic acid fragment to be tested with hybridization buffer (composed of 6 SSC, 5x Denhardt's, 0.5% SDS, 100 µg/l denatured, sonicated salmon sperm DNA) this operation being carried out at 65°C for 1 hour;
- replacement of the hybridization buffer in contact with the support to which the nucleic acid fragment is now bound by hybridization buffer of the same composition and addition of the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) as probe, in particular radioactively labelled, and denatured beforehand;

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- incubation of the said nucleic acid fragment bound to the support in this incubation buffer with the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) at 65°C for a period of about 1 hour;
 - the removal of the buffer containing the probe not bound by two successive washings of 30 minutes each with a buffer solution composed of 2 x SSC and 0.5% SDS at 65°C. --.

Kindly replace the paragraph on page 14, line 24 to line 26 with the following:

-- As examples of DNA or RNA primers according to the invention, mention should be made of the following sequences (SEQ ID NOS: 29 and 30):

3'-5: TTTCGCTAGATCTTGTT & TCTAAATAGAAGAAA --.

Kindly replace the paragraph on page 19, line 10 to line 18 with the following:

-- In fact, as will be described more particularly with the aid of examples of molecules according to the invention in the detailed description which follows, the molecules according to the invention which contain all or part of the amino acid sequence comprised between the positions 200 and 316 shown in Figure 1 (SEQ ID NO: 31), react specifically with the antibodies or the lymphocytes directed against the B and/or T epitopes of the antigens produced at the hepatic stage of P. falciparum, but not with the antibodies directed against other antigens produced by P. falciparum or against antigens produced by other species of Plasmodium. --.

Kindly replace the paragraphs on page 19, line 24 to page 20, line 2 with the following:

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-- These molecules according to the invention comprising all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are not recognized by the former antibodies which react specifically with all or part of the polypeptide defined by the amino acids situated at the positions 200 to 316 in Figure 1.

On the other hand, the polypeptides corresponding to all or part of the peptide sequence shown in figure 3 (SEQ ID NO: 24) are recognized by antibodies which react specifically with antigens localized on the surface of sporozoites (derived from different strains of P. falciparum) as well as with antigens of the hepatic schizonts and the blood schizonts, and finally with the surface of the sporozoites of P. yoelii but not of P. berghei.

It should also be emphasized that the antibodies which recognize specifically the polypeptides corresponding to all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are capable of blocking completely the entry of the sporozoites of P. yoelii into hepatic cells of rodents in vitro, unlike the antibodies directed against the circumsporozoite protein of P. yoelii and of P. falciparum. --.

Kindly replace the paragraph on page 22, at line 22 to line 24 with the following:

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-- As examples of nucleotide probes of the invention, mention should be made of the following sequences:

3'-5' : TTTCGCTAGCTCTTGTT & TCTAAATAGAAGAAA --.

Kindly replace the paragraphs on page 29, line 4 to line 19 with the following:

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-- The insert of 951 base pairs was purified and recloned in the bacteriophage M13 mp19. The DNA sequence and the genomic organization of the LSA gene were then determined. Figure 1 (SEQ ID NO: 31) shows that the clone contains a sequence of 209 amino acids at the 5' end corresponding to a series of 12 repeats of 17 amino acids, similar to that described in the article by Guérin-Marchand et al. (Nature, mentioned above) and then contains a set of 106 amino acids, the structure of which is not repetitive.

As can be seen in Figure 1 (SEQ ID NO: 31), the motif of 17 amino acids is in two repeats (cf. motif corresponding to the positions 35 to 51, and that corresponding to the positions 137 to 153 of figure 1) identical with that described in the article by Guérin-Marchand et al. and the other repeats exhibit a substitution of a leucine by an arginine (cf. positions 8, 59, 76, 110, 127, 161, 178 and 195 of Figure 1) (SEQ ID NO: 31), a substitution of a glutamic acid by an aspartic acid (cf. positions 23 and 91 of Figure 1 (SEQ ID NO: 31)) as well as a substitution of a serine by an arginine (cf. position 205 of Figure 1 (SEQ ID NO: 31)). --.

Please insert the attached paper copy of the "Sequence Listing" between the last page of the Disclosure (page 35) and the first page of the Claims. Please renumber the pages accordingly.